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Research article

REPURPOSING OF FDA APPROVED DRUGS HAVING STRUCTURAL SIMILARITY TO ARTEMISININ AGAINST *Pf*DHFR-TS THROUGH MOLECULAR DOCKING AND MOLECULAR DYNAMICS SIMULATION STUDIES

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Abstract

Background*:Malaria is caused by five species of Plasmodium parasites.It is responsible for causing more than 200 million people malaria positive and kills more than 400,000 people every year. Toxicity, price, bioavailability issues and emergence of drug resistance have doubled down the suitability of the drugs.*

Objective:*To tackle theseproblems, we aimed to identify FDA approved drugs having structural similarities to artemisinin and carried out in-silico based drug repurposing study against Plasmodium falciparum dihydrofolate reductase-thymidylate synthase (PfDHFR-TS).*

Methods:*Similarity search, molecular docking, visualisation of ligand interactions, bioactivity prediction, and molecular dynamics (MD) simulationstechniques were used in the study. The co-crystal inhibitor (RJ1) of PfDHFR-TS was used as the positive control.*

Results and Discussion: *A total of 144 FDA approved drugs were found to have similar chemical structure with artemisinin. Molecular docking revealed 10 drugswith binding affinities higher than RJ1 and theywere subjected to further studies.Tasosartan, exemestane,metolazone, ketazolam and cloxazolamwere removed from the study from the initially selected 10 drugs as they showed poor ligand interactions and poor enzyme inhibitory potential. MD simulations(10ns)revealed that indapamide formed the most*

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stable protein-ligand complex.Indapamide is a thiazide-like diuretic belonging to the class of sulfonamides. The drug has a high binding affinity for PfDHFR-TS, good ligand interactions and good enzyme inhibitory potential.

Conclusion:*We conclude that indapamide has the potential to be repurposed for PfDHFR-TS. Its scaffold may also be used to design and develop newer antimalarial agents.*

Keywords: Molecular docking; Molecular Dynamics; Drug repurposing; Antimalarial; FDA approved drugs.

Introduction

Malaria is a parasitic disease caused by *Plasmodium* parasites that manifests clinically as fever, illness, headache, body ache, tiredness, nausea, vomiting, and sometimes, diarrhoea. Each year, malaria burdens more than 200 million people and kills more than 400,000 people [1]. Many antimalarial drugs have been developed from natural sources as well as through synthetic techniques [2]. However, drug resistance developed by *Plasmodium* parasites against the present antimalarial drugs has greatly affected the efficacy of the antimalarial drugs [3]. In addition to drug resistance, other factors like toxicity, price, and bioavailability issues associated with the present antimalarial drugs have doubled down the drug-resistance problems [4–6].

The concept of drug repurposing has been practically applied for many drugs against several diseases. In drug repurposing, a marketed drug that was initially prescribed for a particular disease was also found to be equally or more effective than the prototype drug for another disease. Drugs such as acetylsalicylic acid, sildenafil, thalidomide, or dimethyl fumarate are classical examples of successfully repurposed drugs [7]. For malaria, several antibiotics have been studied and reported as drugs having activity against *Plasmodium* parasites. Subsequently, these antibiotics were regarded as having the potential to be repurposed for malaria, and as such, antibiotics have been included in many antimalarial treatment regimens [8].

While comparing the chemical structure of the present antimalarial drugs, it can be observed that two individual drugs having similar structures may exert a similar pharmacological action [9]. For example, quinine and its derivatives; and artemisinin and its derivatives showed how two individual drugs having a difference in their structures are still able to exert a similar

biological activity [10]. Auspiciously, it is also widely accepted that different *in-silico* methods can be successfully used to aid the process of drug repurposing [11]. Therefore, in the present study, we aim to identify food and drug administration (FDA) approved drugs that are having structural similarities to artemisinin and carry out *in-silico* based drug repurposing against *Plasmodium falciparum* dihydrofolate reductase-thymidylate synthase (*Pf*DHFR-TS).

Materials and Methods

Retrieval of target protein

The protein thymidylate synthase-dihydrofolate reductase (*TS*-DHFR) is an essential enzyme for folate biosynthesis in *Plasmodium falciparum* [12]. The X-ray crystal structure of Wild-type *Plasmodium falciparum* thymidylate synthase-dihydrofolate reductase (*Pf*DHFR-TS) is selected as the target protein.It has a PDB ID: 3DGA and was downloaded from RSCB-PDB in PDB file format [13]. The target protein is composed of 4 chains *i.e.*, Chain A, Chain B, Chain C and Chain D. The co-crystallised inhibitor (RJ1) was also identified and downloaded in SDF file format. The ligand RJ1 found to have experimental IC_{50}value of 29.9 μM and taken as positive control throughout the study.

Preparation of protein

The preparation of protein was done with the open-source BIOVIA Discovery Studio 2021 software [14]. The Chain A is used for the study and the other remaining chains *i.e.*, Chain B, Chain C and Chain D were deleted from the target protein. The water molecules and heteroatoms were also removed from the target protein. Then the active binding sites are defined with the 'Define and edit binding site' feature of the Discovery Studio software and the active site co-ordinates (x=27.46788; y=5.05044; z=59.30783) were noted and saved for future use. Finally, hydrogen was added to the target protein. The prepared protein was saved in PDB file format for future use.

Preparation of compound library

An online web tool Swiss Similarity was usedto virtually screen several libraries of small molecules to prepare the compound library [15]. The similarity search was achieved with the combined (FP2 fingerprints, Electroshape, Spectrophores, Shape-IT, Align-IT) method using FDA approved drugs as the database to screen drugs having structural similarity toartemisinin. The 2-dimensional (2D) structures of the all the drugs were retrieved from PubChem databaseand saved in SDF file format [16]. Their PubChem compound ID (CID) were also saved.

Energy minimization of ligands

The energy minimisation of the ligands was accomplished with the PyRx 0.8 software [17]. The ligands were minimized by using the default parameters of the PyRx 0.8 virtual screening tool(Force field = Universal Force Field; Optimization algorithm = Conjugate; Total number of steps = 200; Number of steps for update = 1; Stop is energy difference is less than = 0.1). After energy minimization, the ligands were converted to PDBQT file format during which hydrogens were automatically added to the ligands.

Molecular docking simulation studies

For our study, the molecular docking simulation studies (MDSS) were executed with AutoDock Vina on virtual screening tool PyRx 0.8 software [17]. The prepared protein was loaded in the 3D scene in the virtual platform of the software and converted to PDBQT file format when made into macromolecule [18].The original pre-prepared protein was loaded and all the chains except Chain A were removed from the scene. The sequence of the amino acids and the co-crystal ligand were revealed on expanding Chain A. The atoms of co-crystal ligand were labelled to determine the accurate location of the co-crystal inhibitor which is present at the binding site of the protein. In the Vina search of the PyRx 8.0 tool, the pre-defined active binding site co-ordinates were used to adjust to align the 3D affinity grid box so that it covered all the amino acids at the active binding site of the protein. The size of the 3D affinity grid box was kept default at 25 Å and the exhaustiveness at 8. Finally, according to the standard protocols of PyRx tool, the molecular docking simulation studies were carried out [19]. Only 10 FDA approved drugs with the best binding affinity were subjected for further analysis.

Validation of docking protocol

Validation of the docking protocol was carried out by re-docking of the co-crystallized inhibitor (RJ1) to the active binding site of *Pf*DHFR-TS using the same docking protocols as described in the previous sections. The re-docked RJ1- *Pf*DHFR-TS complex was superimposed to the original RJ1-*Pf*DHFR-TS complex. The amino acid sequence alignment was prepared for both complexes and the root mean square deviation (RMSD) between the two complexes was calculated with Discovery Studio Visualizer software 2020 [20]. This study was carried out to access the efficiency and accuracy of the docking protocol used for the study.

Visualization and analysis of ligand interactions

The 2D ligand interactions of 10 FDA approved drugs with the best binding affinities were visualized with Discovery Studio Visualizer software 2021 [14]. The 2D ligand interactions of the original and re-docked RJ1-*Pf*DHFR-TS complexes were also visualized.A drug that does not form any conventional hydrogen bond with the active side residueswas discarded from the study.

Bioactivity prediction

The bioactivity profile of the FDA approved drugs that showed the good ligand interactions were predicted. Molinspiration Chemoinformatics online server tool was used to predict the enzyme inhibitory potential of the FDA approved drugs [21]. The SMILES ID of each FDA approved drugs was identified from PubChem database and these were used to predict the enzyme inhibitory potential [16]. The FDA approved drugs with promising enzyme inhibitory potential was subjected for further studies.

Molecular dynamics simulation studies

The molecular dynamics (MD) simulation studies were used to predict the most stable protein-ligand complex based on the values of root mean square fluctuation (RMSF). A protein-ligand complex is considered to be stable if the RMSF value of all the amino acids is lower than 2.0 Å [22, 23].A complex of each FDA approved drug with *Pf*DHFR-TS was generated with and saved in PDB file format. The MD simulation study was carried out for

the protein itself along with the prepared complexes.The MD simulations were performed on the CABS Flex 2.0 server which utilizes the coarse-grained simulations of protein motion [24].All the parameters of the MD simulation study were kept default (Mode = SS2; Gap =3; Minimum 3.8; Maximum = 8.0; Rigidity = 1.0; Global weight = 1.0; Number of cycles = 50; Cycles between trajectory frames = 50; Temperature of the simulation = 1.4; Time = 10 ns). Restraint parameters such as the solvent probe radius, the minimum atomic radius, the salt radius, and the ionic strength were also kept default. MD simulation studies conducted based on the above parameterswas used to the find the most stable receptor-ligand complex system.The FDA approved drug with most stable protein-ligand complex was identified.

Results and Discussion

Features of the target protein

The crystal structure of the chain A of *Pf*DHFR-TS (Figure 1)wasretrieved from the RCSB-PDB website [13]. It is made up of 4 chains i.e., chain A and chain B (sequence length is 280 for both), chain C and chain D (sequence length is 328). The chain A is complexed with co-crystal inhibitor *viz*. RJ1. RJ1 is also known as 2-{[{[amino(imino)methyl] amino} (imino)methyl] amino}-1-chloro-4-(trifluoromethyl) benzene and bears a PubChem CID of 425841. In the present study, RJ1 was used as a standard drug whenever and wherever necessary.

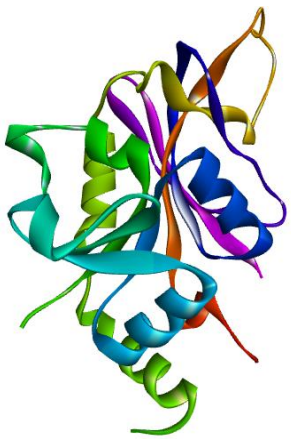

Fig. 1. Crystal structure of the chain A of *Pf*DHFR-TS

Molecular docking simulation studies

Molecular docking is a computational technique that predicts possible interactions between a drug and a protein. It gives an idea of the inhibitory potential of a drug against a protein involved in a disease network [25]. When MDSS is carried out on a PyRx software, it provides a binding affinity value (-kcal/mol) for each ligand so that the binding potential of ligands toward a protein can be ranked [19].

A similarity search on SwissSimilarity web tool revealed that 144 FDA approved drugs has structural similarity with artemisinin.The ligands have a similarity score between 0.0452 to 0.014.A compound library of 144 FDA approved drugs structurally similar to artemisinin to be used in the study were retrieved from the PubChem database. The list of the compound library, their PubChem CID, their similarity score and their binding affinities towards the active binding site of chain A of *Pf*DHFR-TSis given in Table 1. The binding affinity of the RJ1 is also included in Table 1. For each ligand, PyRx software generates a total of 9 poses at the active binding site of the target protein. A more negative binding affinity value suggests a better binding between a compound and a protein [19]. A low binding affinity value also indicates the low energy requirement for protein-ligand binding [26]. In all cases, the first pose is considered the best pose since it has the highest binding affinity towards the target protein. The ninth pose has the lowest binding affinity towards the target protein. Based on this, the first pose and its binding affinity value were considered for the study.

Table 1 PubChem CID, similarity score, and binding affinity of each drug towards the active binding site of *Pf*DHFR-TS

Drug	**PubChem CID**	**Similarity score**	**Binding affinity (-kcal/mol)**
RJI (Standard)	425841	-	7.7
Artemisinin (Standard)	68827	-	9.2
Artemether	68911	0.452	8.6
Ketazolam	33746	0.089	9.6
Flunitrazepam	3380	0.083	9.0
Clopidogrel	60606	0.073	8.1
Nitrendipine	4507	0.072	8.0
Nitrazepam	4506	0.062	8.5
Nilvadipine	4494	0.062	8.6
Clonazepam	2802	0.061	9.0
Methylphenobarbital	8271	0.06	7.5
Nifedipine	4485	0.057	7.8

Phenylbutazone	4781	0.057	8.9
Isocarboxazid	3759	0.054	9.0
Melatonin	896	0.051	7.3
Flumazenil	3373	0.045	8.3
Ruxolitinib	25126798	0.044	9.0
Nisoldipine	4499	0.043	8.6
Alosetron	2099	0.043	9.0
Zaleplon	5719	0.043	8.8
Phenytoin	1775	0.042	8.6
Ethotoin	3292	0.042	7.1
Modafinil	4236	0.041	7.5
Chlormezanone	2717	0.04	7.5
Aminophylline	9433	0.038	2.8
Theophylline	2153	0.038	6.2
Fenofibrate	3339	0.038	8.7
Agomelatine	82148	0.037	8.0
Talbutal	8275	0.037	6.7
Metaxalone	15459	0.037	7.3
Clobazam	2789	0.036	9.0
Hexobarbital	3608	0.036	7.9
Pentobarbital	4737	0.036	6.6
Amobarbital	2164	0.035	6.9
Primidone	4909	0.035	7.6
Etofibrate	65777	0.035	8.1
Prasugrel	6918456	0.035	9.0
Cocaine	446220	0.034	8.1
Procarbazine	4915	0.034	7.0
Articaine	32170	0.034	6.7
Rufinamide	129228	0.033	7.6
Anagrelide	135409400	0.033	7.4
Ramelteon	208902	0.033	7.5
Nevirapine	4463	0.033	8.4
Piroxicam	54676228	0.032	9.0
Glutethimide	3487	0.032	7.2
Metolazone	4170	0.032	9.7
Butalbital	2481	0.032	6.7
Meloxicam	54677470	0.03	8.9
Butethal	6473	0.03	6.7
Butabarbital	2479	0.03	6.6
Methohexital	9034	0.03	7.8
Nitazoxanide	41684	0.029	8.0
Phenindione	4760	0.028	8.4
Nabumetone	4409	0.028	7.9
Carisoprodol	2576	0.028	6.2
Physostigmine	5983	0.027	8.1
Hydralazine	3637	0.027	6.7
Phenacemide	4753	0.027	7.0
Uracil mustard	6194	0.027	5.9
Cloxazolam	2816	0.027	9.4

Tinidazole	5479	0.027	6.2
Bromazepam	2441	0.027	8.3
Lenalidomide	216326	0.026	8.6
Ticlopidine	5472	0.026	7.2
Phensuximide	6839	0.026	7.0
Dolasetron	3033818	0.025	9.7
Methsuximide	6476	0.025	7.1
Secobarbital	5193	0.025	6.9
Tasosartan	60919	0.025	10.8
Milrinone	4197	0.025	7.4
Pirenzepine	4848	0.025	9.8
Delorazepam	17925	0.023	8.9
Ioflupane	10048368	0.023	7.7
Diethylpropion	7029	0.023	6.3
Meprobamate	4064	0.023	5.5
Disopyramide	3114	0.023	7.8
Aprobarbital	6464	0.023	6.5
Pipobroman	4842	0.023	6.3
Monobenzone	7638	0.022	6.9
Zolpidem	5732	0.022	8.7
Enprofylline	1676	0.022	6.4
Thalidomide	5426	0.021	8.7
Lomustine	3950	0.021	6.8
Enoximone	53708	0.021	7.4
Omeprazole	4594	0.021	8.7
Rizatriptan	5078	0.021	7.4
Pilocarpine	5910	0.021	6.2
Levetiracetam	5284583	0.021	5.8
Ibudilast	3671	0.021	7.4
Fenspiride	3344	0.02	8.3
Letrozole	3902	0.02	9.5
Flurazepam	3393	0.02	8.8
Ivacaftor	16220172	0.02	10.2
Praziquantel	4891	0.019	9.7
Phenazopyridine	4756	0.019	7.0
Amrinone	3698	0.019	6.7
Oxcarbazepine	34312	0.019	9.0
Carmustine	2578	0.019	5.3
Benzocaine	2337	0.019	5.8
Clofibrate	2796	0.019	6.5
Remoxipride	54477	0.019	7.7
Indapamide	3702	0.019	9.3
Zileuton	60490	0.019	7.8
Imiquimod	57469	0.019	7.8
Metyrapone	4174	0.019	7.6
Esomeprazole	9568614	0.018	8.7
Dexbrompheniramine	16960	0.018	7.6
Brompheniramine	6834	0.018	7.6
Fenethylline	19527	0.018	8.5

Hydrocodone	5284569	0.017	9.1
Mazindol	4020	0.017	8.9
Pomalidomide	134780	0.017	9.0
Succinylcholine	5314	0.017	5.9
Chlorphenamine	2725	0.017	7.6
Methantheline	4097	0.017	8.4
Loratadine	3957	0.017	10.5
Granisetron	5284566	0.016	9.1
Dacarbazine	135398738	0.016	6.0
Furazolidone	5323714	0.015	6.8
Zonisamide	5734	0.015	6.6
Salicylamide	5147	0.015	5.8
Dexrazoxane	71384	0.015	7.4
Diazepam	3016	0.015	8.6
Methyprylon	4162	0.015	5.7
Methacholine	1993	0.015	4.6
Propantheline	4934	0.015	8.3
Exemestane	60198	0.014	9.7
Aripiprazole	60795	0.014	9.3
Vismodegib	24776445	0.014	9.9
Diltiazem	39186	0.014	8.6
Encainide	48041	0.014	8.6
Alizapride	135413504	0.014	7.9
Methazolamide	4100	0.014	6.5
Cyclizine	6726	0.014	8.2
Theobromine	5429	0.014	6.1
Pentoxifylline	4740	0.014	7.3
Fludiazepam	3369	0.014	9.0
Alogliptin	11450633	0.014	9.1
Pheniramine	4761	0.014	7.3
Procaine	4914	0.014	6.1
Diethylcarbamazine	3052	0.014	5.8
Ketobemidone	10101	0.014	7.1
Isopropamide	3775	0.014	8.6
Brimonidine	2435	0.014	7.7

Among the 144 FDA approved drugs, 10 drugs with the best binding affinities towards chain A of *Pf*DHFR-TSincludestasosartan (-10.8 kcal/mol), loratadine (-10.5 kcal/mol), ivacaftor (-10.2 kcal/mol), vismodegib (-9.9 kcal/mol), pirenzepine (-9.8 kcal/mol), exemestane (-9.7 kcal/mol), metolazone (-9.7 kcal/mol), dolasetron (-9.7 kcal/mol), ketazolam (-9.6 kcal/mol), letrozole (-9.5 kcal/mol), cloxazolam (-9.4 kcal/mol), indapamide (-9.3 kcal/mol). All 10 drugs have better binding affinity towards *Pf*DHFR-TS than RJ1 and artemisinin.

Validation of docking

Validation of docking was carried out to validate the efficiency of the docking procedure. This process was done through re-docking of RJ1 to its active binding site followed by preparation of sequence alignment, superimposition, and RMSD calculation [20].Re-docking of RJ1 to the active binding site of the protein with the PyRx 0.8 virtual screening platform and the AutoDock Vina package was carried out as per the methods previously described. The superimposed structure of original co-crystallized complex with the re-docked co-crystallized complex is given in Figure 2.An RMSD of 0.000 Å was obtained as the output. With this approach, we validate that the docking protocol adopted for the study allowed the re-docked ligands to bind to the similar active binding pocket to which it was originally docked.

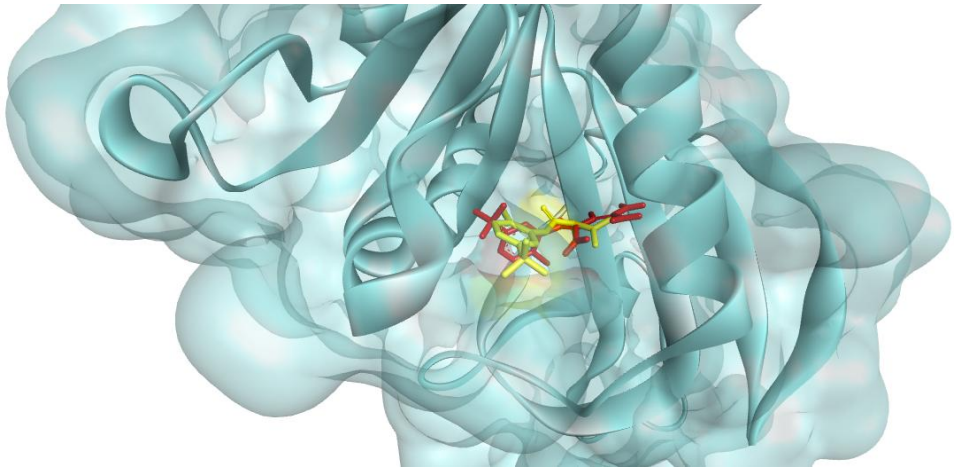

Fig. 2. Re-docking of RJ1 (red color) binds to the same active binding pocket just like the original pose of RJ1 (yellow color).

Visualization and analysis of ligand interactions

Visualization and analysis of the 2D ligand interactions were done with Discovery Studio Visualizer software. The images of the 2D ligand interactions of the FDA approved drugs are given in Figure 3 and Figure 4. A summary of the ligand interactions of each drug is also given in Table 2. The ligand interactions for the original pose of RJ1 along with the re-docked pose of RJ1 is also given in Figures 3 and 4, along with Table 2.

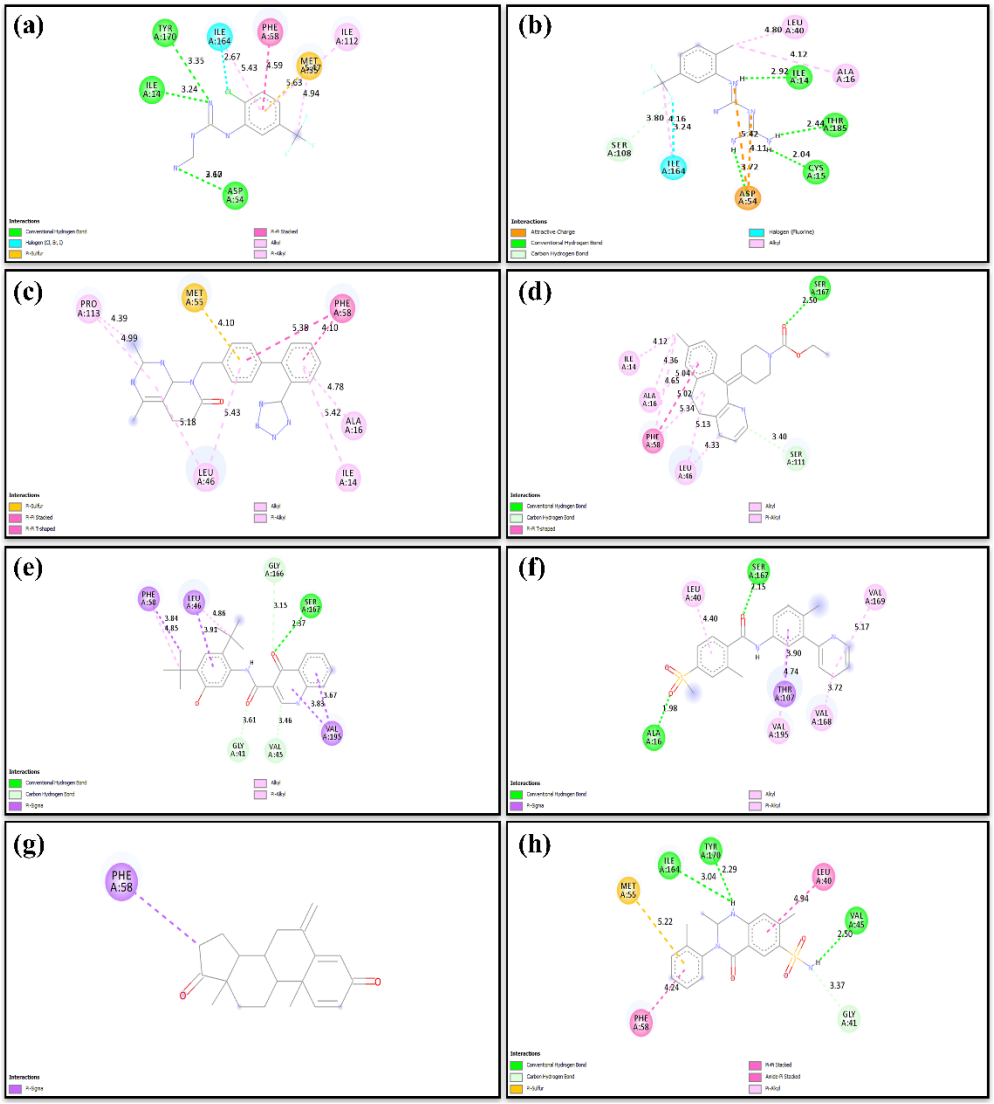

Fig. 3. Visualization of2D ligand interactions of (a) Original binding pose of RJ1; (b) Re-docked pose of RJ1; (c) Tasosartan; (d) Loratadine; (e) Ivacaftor; (f) Vismodegib; (g) Exemestane; and (h) Metolazone

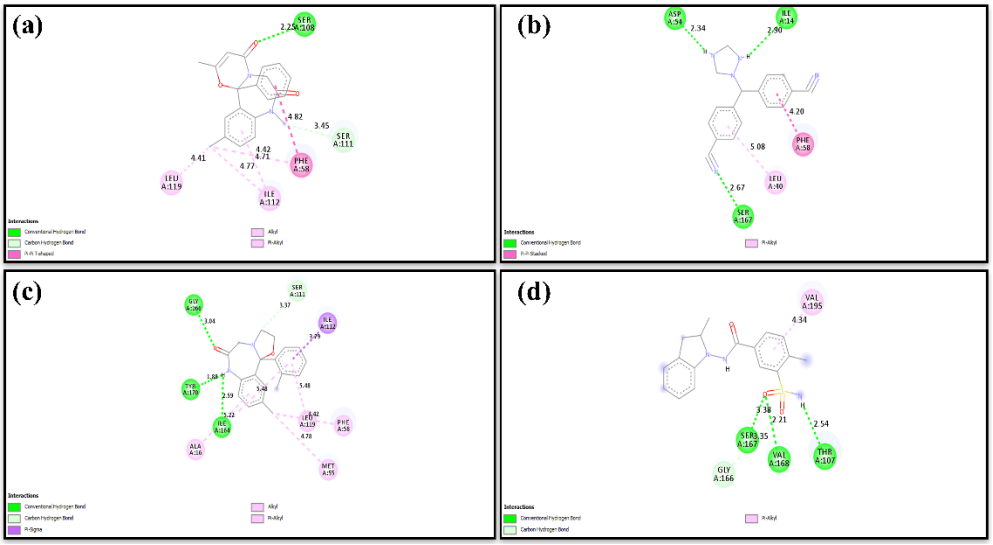

Fig. 4. Visualization of2D ligand interactions of (a) Ketazolam; (b) Letrozole; (c) Cloxazolam; (d) Indapamide

Table 2 Summary ofligand interactions

Drug	Conventional hydrogen bond	Other interacting active site residues
RJ1 original pose	ILE14, ASP54, TYR170	MET55, PHE58, ILE112, ILE164,
RJ1 re-docked pose	ILE14, CYS15, THR185	ALA16, LEU40, ASP54, SER108, ILE164,
Tasosartan	-	ILE14, ALA16, LEU46, MET55, PHE58, PRO113
Loratadine	SER167	ILE14, ALA16, LEU46, PHE58, SER111,
Ivacaftor	SER167	GLY41, VAL45, LEU46, PHE58, GLY166, VAL195
Vismodegib	ALA16, SER167	LEU40, THR107, VAL168, VAL169, VAL196
Exemestane	-	PHE58
Metolazone	VAL45, ILE164, TYR170	LEU40, GLY41, MET55, PHE58,
Ketazolam	SER108	PHE58, SER111, ILE112, LEU119
Letrozole	ILE14, ASP54, SER167	LEU40, PHE58
Cloxazolam	ILE164, GLY166, TYR170	ALA16, MET55, PHE58, SER111, ILE112, LEU119

Indapamide	THR107, VAL168	SER167,	GLY166, VAL195

From these study, it can be observed that majority of the drugs were able to form conventional hydrogen bonds with the active site residues of *Pf*DHFR-TS (Figures 3 and 4, Table 2). However, Tasosartan and exemestane did not form any conventional hydrogen bond with any of the active site residues.

Hydrogen bonds are one of the important factors that plays an important role in the molecular recognition of a drug by a target protein. Simply put, hydrogen bonds are necessary for the interactions between a protein and a ligand [27]. Therefore, it was decided that tasosartan and exemestane should be removed from the study as they did not form any hydrogen bond with the target protein. The rest of the FDA approved drugs were subjected for further analysis.

Bioactivity prediction

After it was validated that the FDA approved drugs were able to produce good ligand interactions, they were further evaluated for the potential to inhibit enzymes with the Molinspiration Chemoinformatics web tool and the results are given in Table 3. This study was carried out as an additional filter to remove drugs with lesser enzyme inhibitory potential. This study will help us to narrow down our options as we aim to find a drug that will have the most promising activity against *Pf*DHFR-TS.

Table 3 Predictedenzyme inhibitory potential

Drug	**Enzyme inhibition**
RJ1	0.01
Loratadine	0.52
Ivacaftor	0.07
Vismodegib	0.11
Metolazone	-0.6
Ketazolam	-0.06
Letrozole	0.30
Cloxazolam	-0.18

Indapamide	0.03

Metolazone (-0.6), ketazolam (-0.06) and cloxazolam (-0.18) showed a negative value during the study for predicting their enzyme inhibitory potential. The rest of the drugs showed a better enzyme inhibitory potential value higher than RJ1 (0.01). Therefore, metolazone, ketazolam and cloxazolam were removed from the study. The rest of the FDA approved drugs were taken for further studies.

Molecular DynamicsSimulation Studies

The MD simulation studies adopted for the present study generates the RMSF valuesfor each amino acid residue thereby providing an idea onthe stability of each amino acid under a given set of parameters for a period of 10 ns [22, 23].This study is needed to validate the conformational stability of the *Pf*DHFR-TS-drug complexes. For each amino acid, a low RMSF value and a high RMSF value indicates limited flexibility and high flexibility in a given system, respectively [23].

For the *Pf*DHFR-TS protein without the presence of any ligand, the RMSF value of a total of 89.49% amino acids was found to be lower than 2Å. For *Pf*DHFR-TS-indapamide complex, the RMSF value of a total of 93.60% amino acids was found to be lower than 2Å. For *Pf*DHFR-TS-ivacaftor complex, the RMSF value of a total of 91.78% amino acids was found to be lower than 2Å. For *Pf*DHFR-TS-letrozole complex, the RMSF value of a total of 89.49% amino acidswas found to be lower than 2Å. For *Pf*DHFR-TS-loratadine complex, the RMSF value of a total of 84.01% amino acids was found to be lower than 2Å. For *Pf*DHFR-TS-vismodegib complex, the RMSF value of a total of 90.86% amino acids was found to be lower than 2Å. The RMSF plot of the amino acids of *Pf*DHFR-TS, both alone and in complex with all the drugs is given in Figure 5.

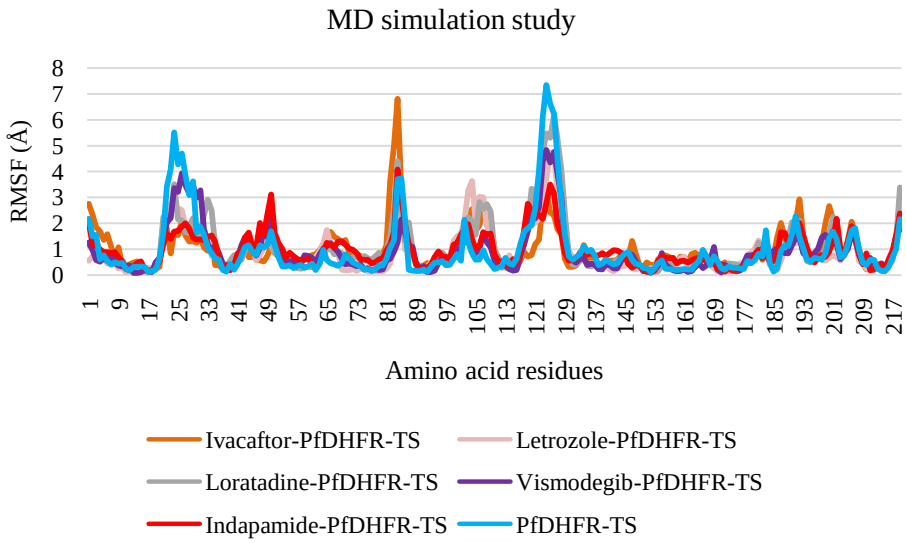

Fig. 5. RMSF (Å) fluctuation plot of all the amino acid residues of chain A of *Pf*DHFR-TS alone and in complex with all the drugs

Among the complexes, *Pf*DHFR-TS-indapamide complex was found to be the most stable complex. It was also observed that all the interacting amino acid residues in *Pf*DHFR-TS-indapamide complex have an RMSF value lesser then 2Å.From the study, it can be observed that the *Pf*DHFR-TS-indapamide complex showed a good conformational stability. The 3D binding pose of indapamide, original and re-docked pose of RJ1 at the active binding site of *Pf*DHFR-TS is given in Figure 6.

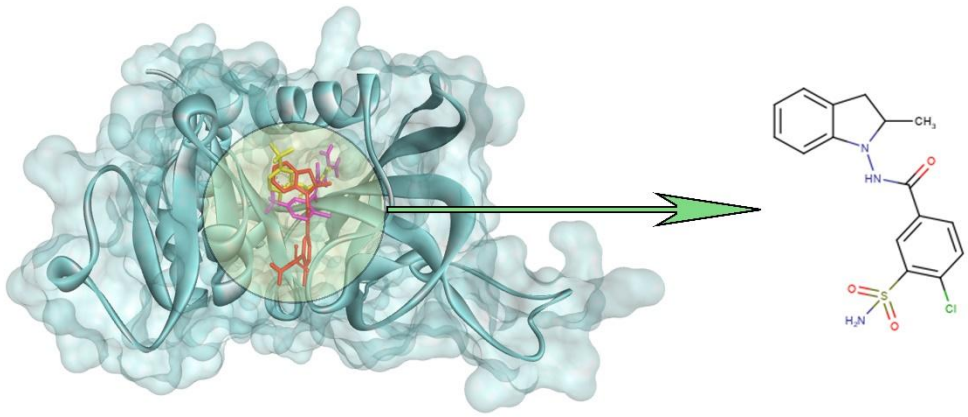

Fig. 6. Structure of indapamide and 3D binding pose of indapamide (red color), original pose (yellow color) and re-docked pose (purple color) of RJ1 at the active binding site of *Pf*DHFR-TS

The superimposition of the stabilized structure of *Pf*DHFR-TS before and after MD simulation is given in Figure7.It can be visually observed that there is minimal difference between the two structures.Calculation of RMSD as described by Shivanikaet al. (2020) revealed that an RMSD value of 3.815 Å existed between the two superimposed structures [20].

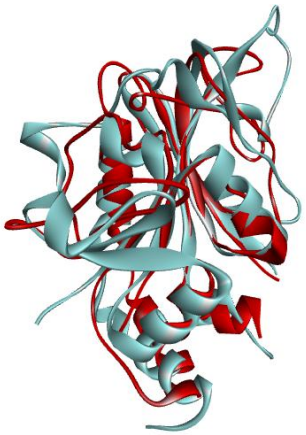

Fig. 7. Superimposition of the stabilized structure of *Pf*DHFR-TS before (pale cyan color) and after MD simulation (red color)

Indapamide is a thiazide like diuretic drug, generally used in the treatment of hypertension, as well as decompensated heart failure. It is structurally similar and also belonged to a class of antimalarial drugs known as sulfonamides.Interestingly, sulfonamides such as sulfadoxine, sulfamethopyrzine, dapsone showed good antimalarial activity in the erythrocytic phase of the parasites. Sulfonamides are specific inhibitors of enzyme dihydropteroate synthetase in tetrahydrofolate synthesis pathway of malarial parasites. As the drugs are structural analogues of para-amino benzoic acid (PABA), they compete with PABA to block its conversion to dihydrofolic acid [28]. As indapamide belonged to the class of sulfonamides that were reported to show good antimalarial activity, the findings of our study aligned with the previous report on sulfonamides.

Conclusion

We conclude that indapamide has the potential to be repurposed for *Pf*DHFR-TS. Its scaffold may be used to design and develop newer antimalarial agents. However, we do acknowledge that the present work is limited to *in-silico* studies. Therefore, further studies (*in-vitro* / *in-vivo*) are required to fully understand the inhibitory potential of indapamide against *Pf*DHFR-TS.

Declarations

Conflict of interest

The authors declare no conflicting interest.

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Author's contributions

JHZ prepared the study design. LP, MS, and JHZ carried out molecular docking simulation studies, post-docking analysis and bioactivity prediction. JHZ carried out validation of docking, molecular dynamics simulation studies and editing of figures. LP and MS carried out drafting of the whole manuscript andprepared the manuscript according to the journal guidelines. DC supervised the study, critically reviewed the paper and provided additional inputs. All four authors read and approved the manuscript.

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